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Comparative evaluation of the levels of fibroblast growth factor-2 prepared from Advanced Platelet Rich Fibrin (A-PRF) in diabetic patients and healthy subjects in Saudi Arabia

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ABSTRACT

Background: Recently, attempts have been made to alter the centrifugation time in platelet-rich fibrin (PRF) preparation protocols; it was believed that low-speed centrifugation (LSCC) improves growth factor release via PRF scaffolds. Diabetes mellitus is a serious, chronic human metabolic disease that has a tremendous impact on patients, families, and society. The objective of this study was to compare the levels of fibroblast growth factor-2 in advanced platelet-rich fibrin (A-PRF) in diabetic patients and those of healthy subjects from Saudi Arabia. *Methods*: Blood samples were obtained from diabetic patients at Riyadh Elm University, Saudi Arabia. Eligibility for the participants to be involved in this study was assessed. Samples were obtained from 38 volunteers, 30 patients with type1 and type 2 diabetes mellitus (test group), and eight non-diabetic volunteers (control group). Blood was extracted using 10 mL syringes and then processed using advanced platelet-rich fibrin (A-PRF) centrifugation. A total of 10 milliliters of complete blood without anticoagulant was centrifuged at 1300 rpm (~200 × *g*) for 14 min at 21–30 °C. *Results*: Measurements done at four different times (Days 1, 7, 14, and Day 28) revealed that the amount of FGF-2 released from A-PRF was significantly lesser among diabetes mellitus patients than in healthy volunteers. *Conclusions*: Compared to the non-diabetic individuals, a decrease in the FGF-2 level released from APRF in diabetic patients was observed.

Keywords: diabetes mellitus, fibroblast growth factor, periodontitis, platelet-rich fibrin

1. INTRODUCTION

Periodontitis is an immune-inflammatory condition involving the structure of the periodontium. It destroys both soft and hard tissue. Clinical periodontal attachment and alveolar bone losses are pathognomonic changes. The initial manifestation of the disease is tooth loss, which is considered morbidity associated with the complex disease process. Several local and systemic factors influence periodontitis. Diabetes is one of the systemic factors affecting the periodontal disease outcome. Conversely, periodontal disease control has an impact on diabetes management (Löe, 1993). Among the systemic factors, diabetes is a major modifiable systemic risk factor that affects the periodontal disease severity and progression (Tan et al., 2006). The relationship between periodontitis and diabetes is well known. Glycation end products cause several changes in the oral microenvironment, which, in turn, influences periodontal health. Changes in oral microflora, host inflammatory cells, and vasculature might initiate periodontitis progress. Prognosis of untreated or uncontrolled diabetics with periodontitis depends on the control of both the diseases and depending upon the severity of both the diseases, there is possibility of alteration in the organ systems (AlJehani, 2014).

Kingdom of Saudi Arabia (KSA) is the biggest nation in the Middle East. Its current population exceeds 33.3 million, and the occurrence of diabetes mellitus in this nation is nearly comparable to that of several developed countries (United Nations, 2017). According to a report of the International Diabetes Federation (IDF), about 25% of the diabetics in the Middle East and North Africa (MENA) reside in KSA. According to the 2017 IDF registry, about 35,000 people in the 10–19 years age group are afflicted with diabetes in this region with the highest number of new cases reaching about 3900. Moreover, first cousin marriages, endogamy, and consanguinity significantly contribute to the alarming increase in the incidence of diabetes in the children of this region (Zayed et al., 2016; Al-Herbish et al., 2008; Warsy et al., 2014; El-Mouzan et al., 2007).

According to the Saudi health ministry's report, approximately 0.9 million people were diagnosed with diabetes mellitus in 1992. By 2010; however, this number had increased to 2.5 million. Hence, the incidence had increased 2.7-fold within two decades. According to a published report, about 4,660 patients with diabetes attended family and medical clinics in 2015 (The Ministry of Health, 2015). These data indicate the increasing burden of diabetes in KSA. Numerous factors are associated with the pathogenesis of diabetes complications. Several animal experiments conducted over the past five years have shown that glucose-dependent processes are affected by platelet-derived growth factor (PDGF), transforming growth factor (TGF), epidermal growth factor (EGF), and others. Hyperglycemia directly enhances platelet reactivity and promotes platelet protein glycation. Hypertriglyceridemia also increases platelet reactivity. As insulin antagonizes platelet activation, Platelet reactivity is predicted to increase with relative or absolute insulin insufficiency (Langham et al., 2003).

Platelet-rich fibrin (PRF), first introduced by Choukroun et al., (2001) was considered as a second-generation platelet concentrate. PRF is composed of various molecular agents and has a variety of medical and dental applications (Choukroun et al., 2006). Within ≤ 7 days of its preparation, it becomes a dense fibrin network comprising leukocytes, cytokines, structural glycoproteins, platelet-derived growth factor, transforming growth factor β-1, vascular endothelial growth factor, and glycoproteins such as thrombospondin-1(Stellos et al., 2010). Fibroblast growth factor (FGF) released from advanced PRF (A-PRF) is one of several similar growth factors that can bind heparin. They induce angiogenesis and endothelial cell mitogenesis. Two subtypes of FGF have been identified, acidic fibroblast growth factor (AFGF) and basic fibroblast growth factor (BFGF), each of which may be derived from a single gene. The seven identified FGFs are affiliates of the heparin-binding growth factor family. They share similar activity and mitogenicity. FGF-2 is a bFGF subtype originally obtained from the brain and the pituitary gland and identified as FGFs. FGF-2 exerts various biological functions on different cell types (Ornitz and Itoh, 2015). It stimulates neuroepithelial cell proliferation and their differentiation into mature neurons and glia (Murphy et al., 1990). FGF- 2 stimulates embryonic limb myogenic cell migration (Webb et al., 1997). Moreover, FGF-2 affects both osteogenesis and bone regeneration (Lisignoli et al., 2001). Based on its angiogenic potential, FGF-2 is a promising candidate for the development of novel therapeutic agents (Presta et al., 2018).

FGF may potentially influence tissue repair and regeneration. FGF was first recognized as a protein that promotes fibroblast proliferation. The FGF family is now known to encompass 22 affiliates and exercise numerous tasks by binding and activating FGF receptors (FGFRs). The RAS/MAP kinase-signaling pathway is stimulated by FGFRs. The clinical implications of PRF are as follows: PRF and PRF membranes, when used in conjunction with bone grafts, expedite healing in lateral sinus floor elevation procedures (Choukroun et al., 2006). PRF has been used to guard and stabilize graft materials during ridge augmentation procedures (Kassolis et al., 2000). After tooth extraction or avulsion, it is used for socket preservation (Toffler et al., 2009). Moreover, PRF membrane also prescribed for root coverage in single- and multiple-tooth recession (Li et al., 2013). In addition, PRF is used in regenerative procedures for treating three- walled osseous defects (Li et al., 2013) and combined periodontal and endodontic

lesions (Hotwani and Sharma, 2014). PRF can also be utilized to treat furcation problems (Sharma and Pradeep, 2011). Furthermore, PRF has been shown to enhance palatal wound healing following free gingival grafts (Kulkarni et al., 2014; Jain et al., 2012).

PRF has several advantages (Davis et al., 2014). It does not require the use of anticoagulants. It promotes slow natural polymerization of fibrin membrane. It induces the formation of a 3D fibrin network that forms a matrix retaining cytokines for extended periods. PRF membrane is highly elastic and flexible. PRF is simple and cost-effective. Purpose of this study was to compare the levels of FGF-2 in A-PRF from Saudi diabetic patients with those of healthy subjects.

2. MATERIALS AND METHODS

Study population and enrollment criteria

Blood samples were collected from type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM) patients and non-diabetic dental patients at Riyadh Elm University, Saudi Arabia. Medical and dental histories of the candidates were retrieved and interviews and clinical examinations were performed in order to ascertain the eligibility of the prospective participants for this study. Recruitment of the study participants and data collection was stared in June 2019 to February 2020 then completed during June-August 2020. The data collection was paused due to the pandemic lockdown from March 2020 to May 2020.

Reliability and validity

Randomization was achieved by soliciting the voluntary participation of diabetic patients at the three campuses of Riyadh Elm University. Positive responses were matched with the study criteria and the final participant sampling group was determined. A trained person extracted blood from the participants while another centrifuged the samples. The data, read and recorded by two independent researchers, were tested for inter-rater and intra-rater reliability by Cohen's κ test.

Ethical approval

This study was approved by the human subject's ethics board of Research Center of Riyadh Elm University under registration/IRB approval No. FPGRP/2019/451/92/95 and was carried out in line with the 1975 Helsinki Declaration of 1975, as amended in 2013.

Inclusion criteria for diabetic patients

Following criteria were applied. Saudi diabetic patients of both genders at Riyadh Elm University within the age group of 24–45 years who voluntarily participated in this study and were not engaged in any other research were included. Controlled and uncontrolled HbA1c diabetics (controlled: < 7; uncontrolled: ≥7) were included.

Exclusion criteria for diabetic patients

Following conditions were used to exclude participants from the study: Refusal to sign the consent form, bleeding disorders and anticoagulant use, pregnancy, tobacco smoking or chewing, non-Saudi origin, hypertension, recipient of chemotherapy and/or radiation therapy.

Inclusion criteria for non-diabetic subjects

Patients at Riyadh Elm University with documented medical history and who agreed to participate in the research and were not engaged in any other study were included.

Exclusion criteria for non-diabetic subjects

Following conditions were used to exclude participants from the study: Refusal to sign the consent form, bleeding disorders, under medication or use of medication with known effect on blood volume in last 6 months, tobacco smoking, diabetes, pregnancy, or lactation.

Sample size calculation

To determine the needed sample size, the effect size and standard deviations were taken from the study of Fujioka-Kobayashi et al., (2017). The needed sample size was calculated using G*Power software (Version 3.1.9.7), with an alpha error of 0.05, a power of 0.95, and an estimated effect size of 0.5, yielding a minimum sample size of 24. However, 38 subjects were selected (diabetics=30, non-diabetics=8) to avoid sample loss throughout the investigation.

Preparation of PRF

Thirty-eight blood samples were obtained, including 30 from T2DM & T1DM patients (test group) and eight from non-diabetic subjects (control group). Blood was extracted with a 10-mL syringe and processed by A-PRF centrifugation. Ten milliliters of a complete blood sample without any anticoagulant was centrifuged at ~200 × g for 14 min at 21–30 °C using a HERMLE Z 206 A centrifuge (Hermle Labortechnik GmbH, Wehingen, Germany). Fibrin clots were then collected from the top layers in the centrifuge tubes and placed onto petri dishes. To each sample, 5 mL Dulbecco's Modified Eagle Medium (DMEM) (SPD Scientific, Petaling Jaya, Selangor, Malaysia) was added. The PRF kit and centrifuge tubes used in the present study were provided by PROCESS for PRF (Nice, France) under U.S.F.D.A. registration No. 3007006186.

Protein quantification with Enzyme-Linked Immunosorbent Assay (ELISA)

The ELISA kit (LS bio, Seattle WA 98121) was based on sandwich ELISA technology. An antibody specific to FGF-2 was coated onto the wells of a 96-well plate. The standards and samples were added to the wells and incubated at 22–28 °C for 4 h, following which the wells were washed with buffer (PBS-T: 10 mM phosphate buffer pH 7.4, 150 mM NaCl, and 0.05% Tween 20 (Product No. P3563). A biotin-conjugated antibody specific to FGF-2 was utilized for detection and 3, 3', 5, 5'- tetramethylbenzidine (TMB) substrate was used to visualize horseradish peroxidase (HRP) activity. HRP reacts with TMB to form a blue product that turns yellow after addition of the solution has ceased. The intensity of the yellow color is proportional to the amount of FGF-2 bound on the plate. Absorbance was measured at 450 nm using a microplate reader (ELISA Microplate Reader, BMG LABTECH) and the FGF-2 concentration was calculated based on the standard curve.

Statistical Analyses

Data for the FGF-2 experiments are displayed as mean ± standard deviation or median (range). Data normality was established by the Kolmogorov–Smirnov, and Shapiro–Wilk tests. P <0.05 indicated skewed data. Correlations were determined by the Pearson correlation analysis. Statistical associations of mean FGF-2 over time among diabetic and non-diabetic subjects were determined using an independent t-test. Paired t-tests were also performed to identify mean differences among FGF-2 over time between diabetic and non-diabetic groups. $P < 0.05$ was considered significant and $P < 0.01$ was considered highly significant. Data analyses were performed in Statistical Packages for Social Sciences (SPSS) v. 21 (SPSS, Chicago, IL, USA).

Reliability Analysis

The internal consistency of the study was evaluated by Interclass Correlation Coefficient (ICC). The reliability analysis conducted on four FGF-2 sampling times between days 1 and 28 yielded a value of 0.818 (81.8%), which indicates high internal consistency.

3. RESULTS

We collected blood samples from 38 volunteers at Riyadh Elm University. Of these, 30 were classified in the diabetic (test) group and eight were in the non-diabetic (control) group. Table 1 compares the mean FGF-2 released from A-PRF over time for the diabetic and non-diabetic subjects. Relative to non-diabetic patients, the diabetic subjects presented significantly lower FGF-2 concentrations at four different sampling times during days 1–28 ($P \leq 0.05$).

Table 1 Comparisons between diabetic and non-diabetic subjects according to mean FGF-2 measured overtime

FGF-2 (pg/mL) measurement

Factor	Day 1 Mean ± SD	Day 7 Mean ± SD	Day 14 Mean ± SD	Day 28 Mean ± SD
Groups				
Diabetic	1.46 ± 0.52	1.17 ± 0.78	1.35 ± 1.24	0.51 ± 0.65
Non-diabetic	2.55 ± 0.29	2.59 ± 0.24	2.67 ± 0.43	2.74 ± 0.59
T-*test*	-5.787	-5.071	-2.921	-8.790
P-*value* §	<0.001 **	<0.001 **	0.006 **	<0.001 **

§P- values were calculated by an independent *t*-test. ** Significant at p<0.05 level

The distribution of mean FGF-2 released from A-PRF measured at four different sampling times (days 1, 7, 14, and 28) for diabetic and non-diabetic subjects is illustrated in Figure 1. The FGF-2 released from A-PRF at the four different sampling times was

significantly lower for the diabetic group ($P < 0.05$). Figure 2 shows a trend line of FGF-2 release from A-PRF measured at different times. The mean FGF- 2 measurement for the non-diabetic subjects was significantly lower at day 1 (2.55) but significantly higher at day 28 (2.74). For the diabetic group, the mean FGF-2 released from A-PRF was significantly lower at day 28 (0.51) but significantly higher at day 1 (1.46).

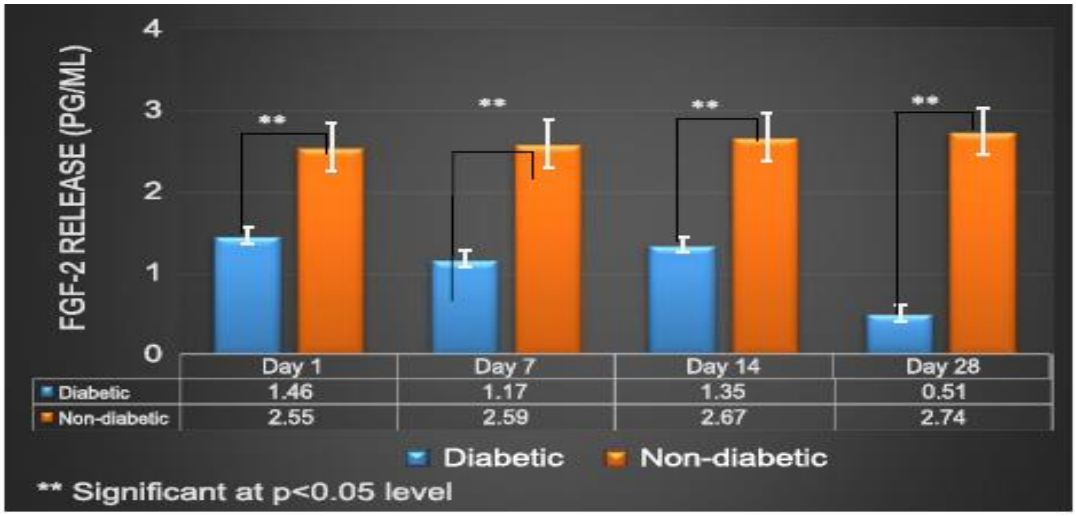

Figure 1 Distribution of mean FGF2 over time for diabetic and non-diabetic subjects

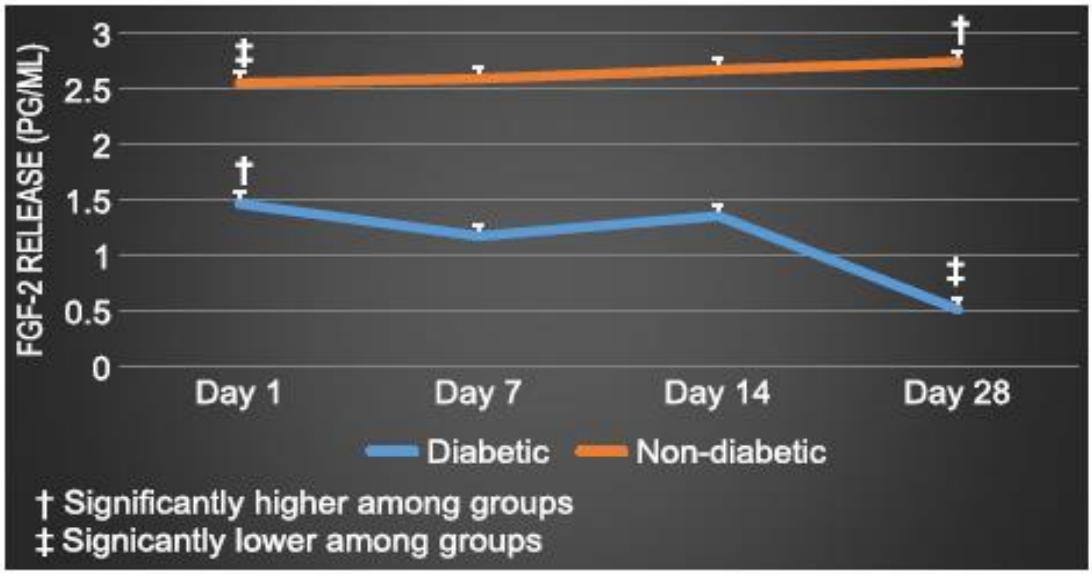

Figure 2 FGF-2 trend line over time for diabetic and non-diabetic subjects

The Pearson correlations among the FGF-2 levels released from A-PRF measured for the diabetic group on days 1, 7, 14, and 28 (Table 2). The correlation was statistically significant between days 14 and 7 ($r= 0.430$, $P < 0.05$). There was also a strong positive correlation between days 28 and 14 ($r = 0.667$; $P < 0.01$). Table 2 shows the Pearson correlation performed on the non-diabetic group. The correlation between days 7 and 1 was highly significant ($r = 0.981$; $P < 0.001$).

Table 2 Pearson's correlation for FGF-2 levels over time between the diabetic and non-diabetic groups (n = 30)

Group	FGF-2	Day 1	Day 7	Day 14	Day 28
Diabetic	Day 1	1			
	Day 7	0.276	1		
	Day 14	-0.034	0.430 *	1	
	Day 28	-0.004	0.230	0.667 **	1
Non-Diabetic	Day 1	1			
	Day 7	0.981 **	1		
	Day 14	-0.251	-0.301	1	
	Day 28	-0.302	0.589	0.632	1

Table 3 Paired t-test for FGF-2 levels over time between the diabetic and non-diabetic groups (n = 30)

	FGF-2	Mean differences	Mean standard error	95% CI of the difference	*P*
Diabetic	Day 1 vs. Day 7	0.285	0.146	-0.013 – 0.583	0.06
	Day 1 vs. Day 14	0.105	0.248	-0.402 – 0.612	0.675
	Day 1 vs. Day 28	0.949	0.151	0.639 – 1.257	<0.001 **
	Day 7 vs. Day 14	-0.179	0.209	-0.607 – 0.248	0.397
	Day 7 vs. Day 28	0.664	0.162	0.333 – 0.995	<0.001 **
	Day 14 vs. Day 28	0.844	0.172	0.492 – 1.195	<0.001 **
Non-Diabetic	Day 1 vs. Day 7	-0.038	0.027	-0.102 – 0.028	0.216
	Day 1 vs. Day 14	-0.117	0.207	-0.606 – 0.371	0.588
	Day 1 vs. Day 28	-0.192	0.259	-0.806 – 0.421	0.483
	Day 7 vs. Day 14	-0.079	0.196	-0.543 – 0.384	0.696
	Day 7 vs. Day 28	-0.155	0.241	-0.726 – 0.416	0.541
	Day 14 vs. Day 28	-0.075	0.162	-0.459 – 0.309	0.657

** Significant at P<0.05 level

Table 3 shows a paired t-test run to evaluate the mean differences in FGF-2 released from A-PRF measured over time for the diabetic group. The paired t-test between days 1 and 28 was highly significant ($P < 0.001$). There was also a highly significant difference between days 7 and 28 ($P < 0.001$) and between days 14 and 28 ($P < 0.001$). Table 3 shows that the paired t-test revealed no significant difference in the FGF-2 released from A-PRF over time for the non-diabetic group.

The distribution of participants according to gender showed that 55.3% male and 44.7% female participants. Based on age, about two-thirds of them (65.8%) were < 35 years of age, whereas 34.2% were ≥ 35 years of age. The distribution of participants in terms of DM type, T1DM and T2DM were equally distributed among the 30 DM patients, while 36.7% of the DM patients presented with uncontrolled HbA1c while 63.3% had controlled HbA1c levels.

4. DISCUSSION

Periodontal regeneration is a major objective of periodontal therapy. Two decades ago, wound healing in periodontal regeneration was investigated via numerous animal experiments and clinical trials. Various novel trends in periodontal regeneration have been explored and assessed, but their results and clinical outcomes are mixed. The drawbacks associated with certain regenerative

models and materials have encouraged researchers to examine different new therapeutic techniques and methods. Hence there is a continual appraisal in the periodontal regeneration therapy (Rojas et al., 2019). Platelet concentrate has attracted interest among practitioners of regenerative science as it has several advantages over other currently available regenerative materials. Its production entails simple blood collection and centrifugation, and it has become a common adjunct for tissue regeneration in several surgical fields, including periodontal regenerative medicine (Shah et al., 2017).

To the best of our understanding, this study is the first of its kind to evaluate the impact of diabetes on PRF and especially on FGF. Both Type 1 and Type 2 diabetes mellitus patients were included in the current study, although both conditions have different etiology, pathogenesis, clinical outcome, and severity. Comparing the relative impact of diabetes type on PRF-induced growth factors was not an objective of the current study. Hence, including both types of diabetes to evaluate the main outcome is justified (Canalis et al., 1988; Peck et al., 2016). The current study consisted of thirty diabetic patients and eight non-diabetic individuals, considering adequate power necessary for interpreting results. The study participant's ages ranged from 24 to 49 years old. The age range was kept as broad as feasible to include as many aged persons as possible, with care given to prevent the influence of aging on the growth factors. The age range was appropriate for interpreting the results since both the diabetic and non-diabetic groups were in the same age range. As a result, the likely deteriorating impact of age in one group or the improved effect of aging in the younger group was averted.

It must be mentioned that the blood sample was measured in four intervals throughout a month to assess FGF-2. The evaluation of the growth factor in this period and intervals contributes to minimizing the one-time alterations found in FGF-2. It also helps to evaluate and prevent the unique influence on the PRF growth factor of one-day cellular transformations. The inflammatory cascade, the rate of cytokines, and the amount of growth can be changed depending on the metabolism. All these obstacles were overcome throughout the four-day evaluation (Saito et al., 2005). This study results indicated significantly lower levels of FGF-2 in diabetic serum than non-diabetic serum at all the tested time intervals (1, 7, 14, and 28 days). Moreover, in diabetic serum mean difference in FGF-2 levels differed significantly between 1 day and 28th day, 7th day and 28th day, and 14th day and 28th day. However, no such mean difference was observed among non-diabetic individuals. It could suggest a possible role of diabetic conditions affecting the production of FGF-2. Moreover, a positive productivity cycle is expected in healthy patients without inflammatory triggers to release a better growth factor (Papageorgiou et al., 2015).

On the contrary, studies have reported a considerable increase in the serum levels of FGF21 in the poorly managed diabetes group than in well-controlled diabetic and healthy controls (Panahi et al., 2016; Mashili et al., 2018). This difference could be due to higher sample sizes and different techniques used in obtaining FGF-2 compared to the present study. Unlike other studies, this study also had several limitations. It took a long time to collect the data due to the lockdowns of the COVID-19 pandemic. The current study was the first to examine FGF2 levels in serum from diabetic and non-diabetic Saudi individuals. This study did not investigate the FGF2 serum levels and other underlying health issues, such as obesity, hypertension, lipid profiles, and systemic diseases, which may directly or indirectly impact FGF-2 production in the body. Smaller samples with fewer people may have influenced the study's findings. To validate the current study results, larger samples and an evaluation of FGF-2 related health factors in diabetes patients should be explored in the future.

5. CONCLUSION

This study found a relative decrease in FGF-2 levels in the diabetic PRF compared to the non-diabetic group. However, there was a consistent increase in the FGF-2 level in the PRF of the non-diabetic group. Comparison of the decrease in FGF-2 in the PRF of the diabetic and non-diabetic groups indicated that diabetes significantly influences FGF-2.

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Authors Contribution

All authors have made substantial contributions to conception and design of the study. HM, HM, OM has been involved in data collection and data analysis. HM, HM, OM have been involved in data interpretation, drafting the manuscript and revising it critically and have given final approval of the version to be published.

Ethical Approval

Ethical approval was obtained from the Research center of Riyadh Elm University, Riyadh Saudi Arabia (FPGRP/2019/451/92/95). The study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2013."

Conflicts of interest

The authors declare that they have no conflict of interest.

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Data and materials availability

All data associated with this study are present in the paper.

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